GUIDELINES FOR USE

PRODUCT: Corning® Matrigel® Basement Membrane Matrix High Concentration Growth Factor Reduced, 10 ml vial

CATALOG NUMBER: 354263

BACKGROUND:

Basement membranes are thin extracellular matrices underlying cells *in vivo*. Corning Matrigel Matrix High Concentration Growth Factor Reduced (GFR) is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in extracellular matrix proteins. Its major component is laminin, followed by collagen IV, heparan sulfate proteoglycans, entactin/nidogen.[1,2] Corning Matrigel Matrix High Concentration GFR also contains TGF-beta, epidermal growth factor, insulin-like growth factor, fibroblast growth factor, tissue plasminogen activator,[3,4] and other growth factors which occur naturally in the EHS tumor. Corning Matrigel Matrix High Concentration GFR is effective for the attachment and differentiation of both normal and transformed anchorage dependent epithelioid and other cell types. These include neurons,[5,6] hepatocytes,[7] Sertoli cells,[8,9] chick lens,[10] and vascular endothelial cells.[11] Corning Matrigel Matrix High Concentration GFR will influence gene expression in adult rat hepatocytes,[12,13] vascular endothelial cells,[14] as well as three dimensional culture in mouse[15-18] and human[19,20] mammary epithelial cells. It is the basis for several types of tumor cell invasion assays,[21,22] will support *in vivo* peripheral nerve regeneration,[23-25] and provides the substrate necessary for the study of angiogenesis both *in vitro*[26,27] and *in vivo*.[25, 28-30] Corning Matrigel Matrix High Concentration GFR also supports *in vivo* propagation of human tumors in immunosupressed mice.[31-33] Corning Matrigel Matrix High Concentration GFR can be used for the transplantation of unsorted mammary cells,[34] as well as sorted epithelial subpopulations embedded in Corning Matrigel Matrix.[35,36] This matrix has also been used as a cancer stem cell model and shown to enhance tumor growth rates *in vivo*.[37]

Corning Matrigel Matrix High Concentration GFR was developed for those who require a reconstituted basement membrane preparation purified and characterized to a greater extent than Corning Matrigel Matrix. The method[38] used to prepare this product effectively reduced the level of a variety of growth factors except for TGF-beta which may be bound to collagen IV[39] and/or sequestered in a latent form that partitions with the major components in the purification procedure. The major components: laminin, collagen IV and entactin (nidogen) are conserved by the process while the level of heparan sulfate proteoglycan is reduced by 40-50%. The following table shows the values for growth factors in Corning Matrigel Matrix compared to a typical lot of Corning Matrigel Matrix High Concentration GFR.

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Parameter	Corning **Matrigel Matrix**	Corning Matrigel Matrix High Concentration GFR
bFGF (pg/mL)[4]	0 - 0.1	0 - 0.1
EGF (ng/mL)	0.5 - 1.3	< 0.5
IGF-1 (ng/mL)	15.6	5
PDGF (pg/mL)	12	< 5
NGF (ng/mL)	< 0.2	< 0.2
TGF-beta (ng/mL)	2.3	1.7
% Protein that gels	80	83

SOURCE: Engelbreth-Holm-Swarm (EHS) Mouse Tumor

FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/mL gentamycin.
Corning® Matrigel® Matrix High Concentration GFR is compatible with all culture media.

STORAGE: Stable when stored at -20°C. Freeze thaws should be minimized by aliquotting into one time use aliquots. Store aliquots in the -20°C freezer until ready for use. **DO NOT STORE IN FROST-FREE FREEZER. KEEP FROZEN**.

EXPIRATION DATE: The expiration date for Corning Matrigel Matrix High Concentration GFR is lot specific and can be found on the product Certificate of Analysis.

CAUTION: It is extremely important that Corning Matrigel Matrix High Concentration GFR and all cultureware or media coming in contact with Corning Matrigel Matrix High Concentration GFR is pre-chilled/ice-cold since Corning Matrigel Matrix High Concentration GFR will start to gel above 10°C. Keep Corning Matrigel Matrix on ice at all times.

RECONSTITUTION AND USE:

Color variations may occur in frozen or thawed vials of Corning Matrigel Matrix High Concentration GFR, ranging from straw yellow to dark red due to the interaction of carbon dioxide with the bicarbonate buffer and phenol red. Variation in color is normal, does not affect product efficacy, and will disappear upon equilibration with 5% CO_2.

Thaw Corning Matrigel Matrix High Concentration GFR by submerging the vial in ice in a 4°C refrigerator, in the back, overnight. Once Corning Matrigel Matrix High Concentration GFR is thawed, swirl vial to ensure that material is evenly dispersed. Keep Corning Matrigel Matrix on ice at all times. Handle with sterile technique. Place thawed vial of Corning Matrigel Matrix High Concentration GFR in sterile area, spray top of vial with 70% ethanol and air dry.

Corning Matrigel Matrix High Concentration GFR may be gently pipetted using a pre-cooled pipet to ensure homogeneity. Aliquot Corning Matrigel Matrix High Concentration GFR to tubes, switching tips whenever Corning Matrigel Matrix High Concentration GFR is

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clogging the tip and/or causing the pipet to measure inaccurately. Gelled Corning® Matrigel® Matrix High Concentration GFR may be re-liquified if placed at 4°C in ice for 24-48 hours.

Corning Matrigel Matrix High Concentration GFR may be used as a thin gel layer (0.5 mm), with cells plated on top. Cells may also be cultured inside the Corning Matrigel Matrix High Concentration GFR, using a 1 mm layer. Extensive dilution will result in a thin, non-gelled protein layer. This may be useful for cell attachment, but may not be as effective in differentiation studies. Corning Matrigel Matrix High Concentration GFR can be used to assess *in vivo* angiogenic activity of different compounds by subcutaneous injection into mice (Corning Matrigel Plug Assay).[2,8,26] The high protein concentration augments the growth of tumors[37-39] and also allows the Corning Matrigel Plug to maintain its integrity after injection. This keeps the injected tumor and/or angiogenic compounds localized for *in situ* analysis and/or future excision.

NOTE: Application specific protocols are posted on the Corning support web page.* The protein concentration for Corning Matrigel Matrix products is lot specific and provided on the Certificate of Analysis. For consistent results dilute Corning Matrigel Matrix products by calculating the specific protein concentration (mg/mL) required. To maintain a gelled consistency we recommend not diluting Corning Matrigel Matrix to less than 3 mg/mL. Use ice-cold serum-free medium to dilute Corning Matrigel Matrix. Ice-cold medium can be added directly to the frozen vial of Corning Matrigel Matrix High Concentration GFR and thawed as recommended in the 'reconstitution and use' section. Mix by pipetting up and down or by swirling the vial in ice.

INJECTION PROTOCOL:

1. It is critical to keep the Corning Matrigel Matrix High Concentration GFR and the Corning Matrigel Matrix/Cell suspension as cold as possible, without freezing, prior to injecting into the mice. It is very important to keep the Corning Matrigel and the Corning Matrigel Matrix/Cell suspension as asceptic as possible throughout the procedure.

2. For each recipient mouse, mix cells ($2x10^5$ or greater) and Corning Matrigel Matrix High Concentration GFR together in a final volume of 0.5 mL on ice.

3. The cells should be in as small a volume as possible. Typically, 250 µl ice cold medium containing $2x10^6$ cells/mL is mixed with 250 µl ice cold Corning Matrigel Matrix High Concentration GFR.

4. Inject the cells subcutaneously in athymic mice using a 19G needle for tissue samples and a 23G needle for cultured cells. The injections should be done quickly to prevent the Corning Matrigel Matrix High Concentration GFR from solidifying.

5. Rotate the syringe when withdrawing to prevent leakage. The needles will need to be changed frequently due to blockage.

NOTE: For more details on this application go to **www.corning.com/lifesciences** to access Document No. CLS-DL-CC-036. (Technical Bulletin 455: Methods for Implantation of Corning Matrigel Matrix into Mice and Tissue Fixation.)

CELL RECOVERY:
Corning Dispase (Cat. No. 354235), Corning Cell Recovery Solution (Cat. No. 354253).

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Most efficient recovery of cells growing on Corning® Matrigel® Matrix High Concentration GFR is accomplished using Corning Cell Recovery Solution that depolymerizes the Corning Matrigel Matrix High Concentration GFR within 7 hours on ice or with Corning Dispase, a metalloenzyme which gently releases the cells allowing for continuous culture.

***NOTE:** For technical resources please visit support page at **www.corning.com/lifesciences**

REFERENCES:

1. Kleinman HK, et al, Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma, *Biochemistry* **21**:6188 (1982).
2. Kleinman HK, et al, Basement membrane complexes with biological activity, *Biochemistry* **25**:312 (1986).
3. Vukicevic S, et al, Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular activity related to extracellular matrix components, *Exp Cell Res* **202**:1 (1992).
4. McGuire PG and Seeds NW, The interaction of plasminogen activator with a reconstituted basement membrane matrix and extracellular macromolecules produced by cultured epithelial cells, *J. Cell. Biochem.* **40**:215 (1989).
5. Biederer T and Scheiffele P, Mixed-culture assays for analyzing neuronal synapse formation, *Nat Protoc* **2**(3):670 (2007).
6. Li Y, et al, Essential Role of TRPC channels in the guidance of nerve growth cones by brain-derived neurotrophic factor, *Nature* **434**:894 (2005).
7. Bi Y, et al, Use of cryopreserved human hepatocytes in sandwich culture to measure hepatobiliary transport, *Drug Metab Dispos.* **34**(9):1658 (2006).
8. Gassei K, et al, Immature rat seminiferous tubules reconstructed *in vitro* express markers of Sertoli cell maturation after xenografting into nude mouse hosts, *Mol Hum Reprod*. **16**(2):97 (2010).
9. Yu X**,** et al, Essential role of extracellular matrix (ECM) overlay in establishing the functional integrity of primary neonatal rat sertoli cell/gonocyte co-cultures: An improved *in vitro* model for assessment of male reproductive toxicity, *Toxicol Sci* **84**(2):378 (2005).
10. Chandrasekher G, and Sailaja D, Differential activation of phosphatidylinositol 3-kinase signaling during proliferation and differentiation of lens epithelial cells, *Invest Ophthalmol Vis Sci.* **44**(10):4400 (2003).
11. McGuire PG, and Orkin RW, A simple procedure to culture and passage endothelial cells from large vessels of small animals, *Biotechniques* **5**(6):456 (1987).
12. Bissel DM, et al, Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver, *J. Clin Invest.* **79**:801 (1987).
13. Page JL, et al, Gene expression profiling of extracellular matrix as an effector of human hepatocyte phenotype in primary cell culture, *Toxicol Sci* **97**(2):384 (2007).
14. Cooley LS, et al, Reversible transdifferentiation of blood vascular endothelial cells to a lymphatic-like phenotype *in vitro*, *J Cell Sci.* **123**(Pt 21):3808 (2010).
15 Li ML, et al, Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells, *Proc. Nat. Acad. Sci. USA* **84**:136 (1987).
16 Barcellof MH, et al, Functional differentiation and aveolar morphogenesis of primary mammary cultures on reconstituted basement membrane, *Development* **105**:223 (1989).
17. Roskelley CD, et al, Extracellular matrix-dependent tissue-specific gene expression in mammary epithelial cells requires both physical and biochemical signal transduction, *Proc. Nat. Acad. Sci. USA* **91**(26):12378 (1994).
18. Xu R, et al, Extracellular matrix-regulated gene expression requires cooperation of SWI/SNF and transcription factors, *J. Biol. Chem.* **282**(20):14992 (2007).
19. Debnath J, et al, Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures, *Methods* **30**(3):256 (2003).
20. Muthuswamy SK, et al, ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini, *Nat. Cell Biol.* **3**(9):785 (2001).
21. Albini A, et al, A rapid *in vitro* assay for quantitating the invasive potential of tumor cells, *Cancer Res,***47**:3239 (1987).
22. Poincloux R, et al, Contractility of the cell rear drives invasion of breast tumor cells in 3D Matrigel, P*roc Natl Acad Sci USA*.**108**(5):1943 (2011).
23. Madison R, et al, Increased rate of peripheral nerve regeneration using bioresorbable nerve guides and laminin containing gel, *Exp. Neurology* **88**:767 (1985).
24. Xu XM, et al, Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord, *J. Comp. Neurol.* **351**(1):145 (1994).
25. Lopatina T, et al, Adipose-derived stem cells stimulate regeneration of peripheral nerves: BDNF secreted by these cells promotes nerve healing and axon growth de novo, *PLoS One* **6**(3):e17899 (2011).
26. Kubota Y, et al, Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures, *J. Cell Biol.* **107**:1589 (1988).
27. Ponce ML, Tube formation: an *in vitro* matrigel angiogenesis assay, *Methods Mol Biol.* **467**:183 (2009).
28. Passaniti A, et al, A simple, quantitative method for assessing angiogenesis and anti-angiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor, *Lab Invest.* **67**:519 (1992).
29. Isaji M, et al, Tranilast inhibits the proliferation, chemotaxis and tube formation of human microvascular endothelial cells *in vitro* and angiogenesis *in vivo*, *Br J Pharmacol* **122**:1061 (1997).
30. Adini A, et al, Matrigel cytometry: a novel method for quantifying angiogenesis *in vivo*, *J Immunol Method*. **342**(1-2):78 (2009).
31. Albini A, et al, Matrigel promotes retinoblastoma cell growth *in vitro* and *in vivo*, *Int. J. Cancer* **52**(2):234 (1992).
32. Yue W, and Brodie A, MCF-7 human breast carcinomas in nude mice as a model for evaluating aromatase inhibitors, *J. Steroid Biochem. Mol. Biol.* **44**(4-6):671 (1993).
33. Angelucci A, et al, Suppression of EGF-R signaling reduces the incidence of prostate cancer metastasis in nude mice, *Endocr-Relat Cancer* **13**(1):197 (2006).

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34. Moraes RC, et al, Constitutive activation of smoothened (SMO) in mammary glands of transgenic mice leads to increased proliferation, altered differentiation and ductal dysplasia, *Development* **134**:1231 (2007).
35. Zeng YA, and Nusse R, Wnt proteins are self-renewal factors for mammary stem cells and promote their long-term expansion in culture, *Cell Stem Cell* **6**:568 (2010).
36. Jeselsohn R, et al, Cyclin D1 kinase activity is required for the self-renewal of mammary stem and progenitor cells that are targets of MMTV-ErbB2 tumorigenesis, *Cancer Cell* **17**:65 (2010).
37. Quintana E, et al, Efficient tumor formation by single human melanoma cells, *Nature* **456**:593 (2008).
38. Anai S, et al, Dual targeting of Bcl-2 and VEGF: a potential strategy to improve therapy for prostate cancer, *Urol Oncol* **29**:421 (2011).
39. Cao J, et al, A subretinal matrigel rat choroidal neovascularization (CNV) model and inhibition of CNV and associated inflammation and fibrosis by VEGF trap, *Invest Ophthalmol Vis Sci* **51**:6009 (2010).

CALIFORNIA PROPOSITION 65 NOTICE

WARNING:	This product contains a chemical known to the state of California to cause cancer.
Component:	**Chloroform**

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